IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **10/007,459**

Applicants : **David L. Lewis et al.**

Filed : **11/07/2001**

Art Unit : **1635**

Examiner : **Gibbs, Terra C.**

Docket No. : **Mirus.030.04**

For: **Inhibition of Gene Expression by Delivery of Small Interfering RNA to Post-Embryonic Animal Cells *In Vivo***

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, David Lewis, hereby declare as follows:

1. I am an inventor of the captioned application.
2. I submit with this Declaration and Response further experimental material (attached) illustrating: (a) effect of volume on increasing permeability in liver tissue following tail vein injection. The experiments were performed according to the methods provided in the Specification.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. David Lewis 7/25/07 date

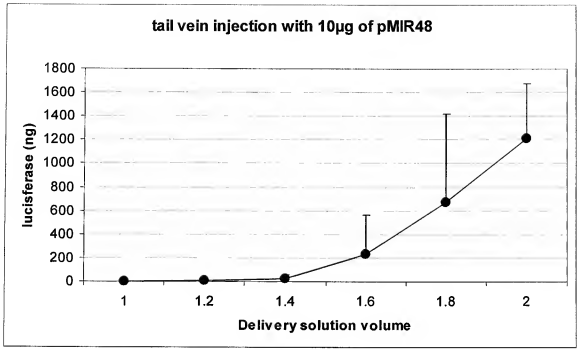
Effect of Injection Volume on Vessel Permeability in Liver Following Tail Vein Injection, as Measured by Nucleic Acid Delivery.

To demonstrate the effect of volume on increasing permeability of vessels in the target tissue, 10 μg nucleic acid (plasmid DNA encoding the luciferase gene (pMIR48)) was injected, in varying volumes, into the tail vein of mice. Increased permeability is evidenced by delivery and expression of the luciferase gene in target tissue liver cells. The experiment was performed in accordance with example 1B of U.S. Application 10/007,459

Delivery of DNA to target liver cells in mice. Plasmid pMIR48 (10μg), containing the luc+ coding region (Promega Corp.) and a chimeric intron downstream of the cytomegalovirus major immediate-early enhancer/promoter was diluted in 1-2 ml (1.0, 1.2, 1.4, 1.6, 1.8, 2.0 ml) Ringer's solution (147mM NaCl, 4mM KCl, 1.13mM $CaCl_2$) and injected into the tail vein in approximately 7 seconds.

As shown in the table and graph below, expression approached zero for volumes less than 1.2 ml (>1800 fold lower than for injection of 2.0 ml). Thus, for injection into the tail vein, volumes less that 1.2 ml did not result in increased permeability of vessels in the liver tissue. Zimmer et al. (Methods 1999) injected 0.125 ml, 8 times lower than the 1.0 ml injection volume shown in this example

	luciferase expression (ng)					
injection volume (ml)	**1**	**1.2**	**1.4**	**1.6**	**1.8**	**2**
average (n=4)	0.64	6.93	25.44	234.11	671.06	1208.55
standard deviation	0.98	12.07	30.90	333.77	744.91	468.53



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